U.S.S.N. 09/779,427

Filed: February 8, 2001

AMENDMENT AND RESPONSE TO OFFICE ACTION

(G) 3-hydroxybutyric acid, 3-hydroxyvaleric acid, 3-hydroxy-heptanoic acid and 4-hydroxyheptanoic acid;

(H) 3-hydroxybutyric acid, 3 hydroxyvaleric acid, 3-hydroxyhexanoic acid, 3-hydroxyoctanoic acid, and 4-hydroxyoctanoic acid;

(I) 3-hydroxybutyric acid, 3-hydroxyhexanoic acid and 4-hydroxyhexanoic acid; and

(J) 3-hydroxybutyric acid, and 5-hydroxyhexanoic acid

Remarks

Claims 1-18 have been amended. Claims 37-42 have been added.

The present invention is directed to a process for the preparation of poly(hydroxy fatty acids) using recombinant bacteria containing at least one fragment of the poly(hydroxy fatty acid) synthase gene from *Thiocapsa pfennigii*. The recombinant bacteria are grown with an appropriate carbon source as a substrate and then the poly(hydroxy fatty acids) are extracted.

Objected to Declaration

An executed supplemental oath/declaration of Dr. Mathias Leibergesell is enclosed in accordance with 37 C.F.R. 1.67(a)(2).

Objections to Specification and Claims

The specification has been amended to correct errors in labeling of plasmid pHP1014::156. Claims 1-18 have been amended to include an article "A" or 'The" at the beginning of the claims to give proper antecedent basis.

Rejection Under 35 U.S.C. § 112, first paragraph

Claims 1-18 were rejected under 35 U.S.C. § 112, first paragraph, as not being enabled. Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

The amended claims no longer recite the limitation of the specific bacterial strains and plasmids including: *Pseudomonas putida* GPp104 (pHP1014::E156), *Pseudomonas putida* GPp104 (pHP1014::B28+), *Alcaligenes eutrophus* PHB 4(pHP1014::E156), and *Alcaligenes eutrophus* PHB 4(pHP1014::B28+).

The claims now clearly define the claimed subject matter as any bacteria encoding and expressing the gene encoding the PHA synthase from *Thiocapsa pfennigi*. The DNA and protein sequences of PHA synthase from *Thiocapsa pfennigi* are disclosed in Figure 1 of the specification. One of skill in the art would be sufficiently enabled to use available methods to express this enzyme in organisms such as *P. putida* and *A. eutrophus* for PHA synthesis.

Methods for growth, addition of the appropriate carbon source and isolation of the polymer are also known in the art. Therefore, the process as defined by the amended claims, is enabled.

Rejection Under 35 U.S.C. § 112, second paragraph

Claims 1-18 were rejected under 35 U.S.C. § 112, second paragraph, as being indefinite. Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

The Examiners suggested amendments to Claims 1-18 have been incorporated to more

AMENDMENT AND RESPONSE TO OFFICE ACTION

Claim I has been amended to remove indefinite terms such as "as the case may be", "its derivatives, "its", and "a certain time". The Markush group of claim I has been amended to clearly list the substrate carbon sources.

Claim 3 has been amended to remove specific reference to "glucose and fructose" and recite only the group "hexoses" that was originally present.

Claims 3 and 18 have been amended to replace the term "a group comprising" to "a group consisting".

Claims 3, 9, 11, 12 and 14 have been amended to remove narrower range limitation and the narrower ranges have been recast as new dependent claims 37-42.

Claims 6, 11, and 12 have been amended to remove the word "preferably".

Claims 7, 12, and 13 have been amended to replace the terms "cultivated"/ "cultivating" with "incubated"/ "incubating".

Claim 9 has been amended to replace the term "uses" with the term "offers" in accordance with claim 1.

Claim 11 has been amended to remove the term "in each case".

Claim 12 has been amended to remove the term "especially".

Claim 14 has been amended to remove the term "and/or".

AMENDMENT AND RESPONSE TO OFFICE ACTION

Allowance of claims 1-18, and 37-42 is respectfully solicited.

Respectfully submitted,

Patrea/L. Pabst

Reg. No. 31,284

Date: December 26, 2002

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Certificate of Mailing Under 37 C.F.R. § 1.8(a)

I hereby certify that this paper, along with any paper referred to as being attached or enclosed, is being deposited with the United States Postal Service on the date shown below with sufficient postage as first-class mail in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

Aisha Wyatt

Date: December 26, 2002

MARKED UP VERSION OF AMENDED SPECIFICATION PARAGRAPHS PURSUANT TO 37 C.F.R. § 1.121

3-hydroxybutyric acid, 3-hydroxyvaleric acid, 3-hydroxy-heptanoic acid and 4-hydroxybutyric acid, 3 hydroxyvaleric acid, 3-hydroxybutyric acid, 3 hydroxyvaleric acid, 3-hydroxybutyric acid, and 4-hydroxyoctanoic acid;

3-hydroxybutyric acid, 3-hydroxyhexanoic acid and 4-hydroxyhexanoic acid; and
3-hydroxybutyric acid, and 5-hydroxyhexanoic acid

Clean Version of Amended Claims

Pursuant to 37 C.F.R. § 1.121(c)(1)(ii)

- 1. (Amended) A process for the preparation of poly(hydroxy fatty acids) comprising incubating a recombinant organism in a mineral medium under aerobic conditions, expressing at least one fragment of the gene encoding the poly(hydroxy fatty acid) synthase from *Thiocapsa pfennigii* with a substrate carbon source, wherein the recombinant organism produces a poly(hydroxy fatty acid) and, the poly (hydroxy fatty acid) is recovered.
- the pory (flydroxy fatty acid) is recovered.
- 2. (Amended) The process of claim 1, wherein the bacteria are pre-cultivated in a complex medium.
- 3. (Amended) The process of claim 1, wherein one also adds to the bacterial culture at least one additional carbon source which promotes growth, whereby the carbon source is selected from the group consisting of

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PURSUANT TO 37 C.F.R. § 1.121

citric acid, citric acid salts, citric acid esters and citric acid lactones, octanoic acid, octanoic acid salts, octanoic acid esters, octanoic acid lactones, gluconic acid, gluconic acid salts, gluconic acid esters, gluconic acid lactones, hexoses, and combinations thereof.

- 4. (Amended) The process of claim 1, wherein the process is carried out in the form of a batch process, a fed-batch process, a two-step process or a continuous flow process.
- 5. (Amended) The process of claim 1, wherein the poly(hydroxy fatty acid) is obtained in a concentration of approximately 15 to 70% by weight based on the dry mass of the bacterial cells.
- 6. (Amended) The process of claim 1, wherein the poly(hydroxy fatty acids) are obtained in the form of copolyesters with at least two subunits.
- 7. (Amended) The process of claim 1, wherein the recombinant bacteria are cultivated at cell densities of up to 100 g of dry cellular mass per liter of bacterial nutrient medium.
- 8. (Amended) The process of claim 1, wherein one offers the substrate carbon source in excess.
- 9. (Amended) The process of claim 8, wherein one offers the substrate carbon source at a concentration of approximately 0.1 to 5% by weight.
- 10. (Amended) The process of claim 9, wherein one increases the concentration of the substrate carbon source in the culture medium in steps, optionally with pre-cultivation in the

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- 11. (Amended) The process of claim 10, wherein, one adds approximately 0.5% (weight/volume) of neutralized substrate carbon source after approximately 12 h and 24 h at approximately 27°C to 35°C.
- 12. (Amended) The process of claim 1, wherein cultivation takes place for approximately 24 h to 96 h.
- 13. (Amended) The process of claim 1, wherein the recombinant bacteria are cultivated under conditions deficient in an element wherein the element is selected from the group consisting of nitrogen, magnesium or phosphate.
- 14. (Amended) The process of claim 1, wherein the harvested recombinant bacteria are broken open in order to obtain the poly(hydroxy fatty acids) that have been produced.
- 15. (Amended) The process of Claim 14, wherein the harvested recombinant bacteria are lyophilized and then extracted with an organic solvent, selected from the group consisting of chloroform or methylene chloride, in order to break open the recombinant bacteria and to obtain the poly(hydroxy fatty acids).
- 16. (Amended) The process of claim 15, wherein the extracted poly(hydroxy fatty acid) produced is precipitated by introducing a hydrophilic solvent, selected from the group consisting of water and a lower alcohol, wherein the product is obtained in essentially pure form by removing the hydrophilic solvent.
 - 17. (Amended) The process of claim 14, wherein the harvested recombinant bacteria

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cocktail, and a combination thereof wherein the bacterial cell grana, which contain the poly(hydroxy fatty acid), sediment to the bottom of the bio-reactor and are collected from there in order to be processed further.

18. (Amended) The process of claim 17, wherein the lytic enzyme cocktail contains enzymes which are selected from the group consisting of

lysozyme; proteases; other hydrolytic enzymes: and combinations thereof.

- 37. The process of claim 1, wherein the poly(hydroxy fatty acid) is obtained in a concentration of approximately 15 to 50% by weight based on the dry mass of the bacterial cells.
- 38. The process of claim 1, wherein the poly(hydroxy fatty acid) is obtained in a concentration of approximately 40% by weight based on the dry mass of the bacterial cells.
- The process of claim 10, wherein, in each case, one adds approximately 0.5% (weight/volume) of neutralized substrate carbon source after approximately 12 h and 24 h at approximately 30°C.
- 40. The process of claim 1, wherein cultivation takes place for approximately 36 h to 72 h.
- 41. The process of claim 1, wherein cultivation takes place for approximately 48 h to 72 h.
- 42. The process of claim 1 wherein the poly(hydroxy fatty acid) polymer is comprised of one or more monomers selected from the group consisting of :

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3-hydroxybutyric acid, 3 hydroxyvaleric acid, 4-hydroxy-valeric acid, 3-hydroxyhexanoic acid and 3-hydroxyoctanoic acid;

3-hydroxybutyric acid, 3-hydroxyhexanoic acid, 5-hydroxyhexanoic acid, and 3-hydroxyoctanoic acid;

3-hydroxybutyric acid, 3 hydroxyvaleric acid, 3-hydroxyhexanoic acid, 3-hydroxyheptanoic acid, 4-hydroxyheptanoic acid and 3-hydroxyoctanoic acid:

3-hydroxybutyric acid, 3 hydroxyhexanoic acid, 3-hydroxy-octanoic acid and 4-hydroxyoctanoic acid;

3-hydroxybutyric acid. 3-hydroxyhexanoic acid and 5-hydroxyhexanoic acid;

3-hydroxybutyric acid, 3-hydroxyvaleric acid, 3-hydroxy-heptanoic acid and 4-hydroxyheptanoic acid;

3-hydroxybutyric acid, 3 hydroxyvaleric acid, 3-hydroxyhexanoic acid, 3-hydroxyoctanoic acid, and 4-hydroxyoctanoic acid;

3-hydroxybutyric acid, 3-hydroxyhexanoic acid and 4-hydroxyhexanoic acid; and 3-hydroxybutyric acid, and 5-hydroxyhexanoic acid

Marked Up Version of Amended Specification Paragraphs MARKED UP VERSION OF AMENDED SPECIFICATION PARAGRAPHS Pursuant to 37 C.F.R. § 1.121(b)(1)(iii)

Please replace the paragraph bridging pages 1 and 2 with the following paragraph.

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PURSUANT TO 37 C.F.R. § 1.121

-- The present invention relates to a process for the production of poly(hydroxyl acids) by means of recombinant bacteria which contain and express at least one fragment of the gene of poly(hydroxy fatty acid) synthase from *Thiocapsa pfennigii* and which are selected from the group comprising: *Pseudomonas putida* GPp104 (pHP1014::E156), *Alcaligenes eutrophus* PHB 4 [(pHP1014::E156)] (pHP1014::E156), *Pseudomonas putida* GPp104 (pHP1014::B28+) [DSM # 9417] and *Alcaligenes eutrophus* PHB 4 (pHP1014:B28+) [DSM # 9418], whereby the bacteria are cultivated in a mineral medium under aerobic conditions, whereby one offers the bacteria at least one substrate carbon source which is selected from the group consisting of: levulinic acid, salts of levulinic acid, esters of levulinic acid, lactones of levulinic acid, substituted levulinic acid or, as the case may be, its derivatives: 5-hydroxyhexanoic acid, its salts, esters and lactones: 4-hydroxyheptanoic acid, its salts, esters and lactones: 4-hydroxyyoctanoic acid, its salts, esters and lactones: 4-hydroxyoctanoic acid, its salts, e

a recombinant bacterial strain characterized by the feature that the bacterial strain is selected from the group which comprises *Pseudomonas putida* GPp104 (pHP1014::B28+) [DSM # 9417] and *Alcaligenes eutrophus* PHB 4 (pHP1014::B28+) [DSM # 9418];

a poly(hydroxyl fatty acid) produced by any one of the previously described processes:

and a DNA fragment which codes for a pha E component and a pha C component of the poly(hydroxyl fatty acid) synthase from *Thiocapsa pfennigii* characterized by the feature that it has at least the nucleotide sequence of sequence sections 180 through 1280 (phaE) and 1322 through 2392 (*phaC*) of the DNA sequence SEQ ID NO:1.--

Please replace the paragraph bridging pages 10 and 11 with the following paragraph.

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bacteria which contain and express at least one fragment of the gene of poly(hydroxy fatty acid) synthase from Thiocapsa pfennigii and which are selected from the group comprising: PHB putida GPp104 (pHP1014::E156), Alcaligenes eutrophus Pseudomonas [(pHP1014::E1S6)] (pHP1014::E156), Pseudomonas putida GPp104 (pHP1014::B28+) [DSM #9417] and Alcaligenes eutrophus PHB 4 (pHP1014:B28+) [DSM # 9418], whereby the bacteria are cultivated in a mineral medium under aerobic conditions, whereby one offers the bacteria at least one substrate carbon source which is selected from the group consisting of: levulinic acid, salts of levulinic acid, esters of levulinic acid, lactones of levulinic acid, substituted levulinic acid or, as the case may be, its derivatives; 5-hydroxyhexanoic acid, its salts, esters and lactones; 4-hydroxyheptanoic acid, its salts, esters and lactones; 4-hydroxyoctanoic acid, its salts, esters and lactones; their halogenated derivatives as well as their mixtures; one incubates the bacteria for a certain time with the carbon; and one isolates the poly(hydroxy fatty acid) polymers that have been synthesized by the bacteria.--

Please replace the paragraph bridging pages 11 and 12 with the following paragraph.

-- In accordance with the process for the preparation of poly(hydroxy fatty acids) with at least one subunit by means of recombinant bacteria which contain and express at least one fragment of the gene of poly(hydroxy fatty acid) synthase from *Thiocapsa pfennigii* and which are selected from the group comprising: *Pseudomonas putida* GPp104 (pHP1014::E156), *Alcaligenes eutrophus* PHB 4 [(pHP1014::E156)] (pHP1014::E156). *Pseudomonas putida* GPp104 (pHP1014::B28+) [DSM # 9417] and *Alcaligenes eutrophus* PHB 4 (pHP1014:B28+) [DSM # 9418], whereby the bacteria are cultivated in a mineral medium under aerobic conditions, whereby one offers the bacteria at least one substrate carbon source which is selected from the group consisting of: levulinic acid, salts of levulinic acid, esters of levulinic acid, lactones of levulinic acid, substituted levulinic acid or, as the case may be, its derivatives: 5-

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well as their mixtures; one incubates the bacteria for a certain time with the carbon source; and one isolates the poly(hydroxy fatty acid) polymers that have been synthesized by the bacteria, it has been possible for the first time to produce 4HV-containing polyesters starting out from levulinic acid. The chemical structure of levulinic acid is reproduced in the following formula:--

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PURSUANT TO 37 C.F.R. § 1.121

Marked Up Version of Amended Claims Pursuant to 37 C.F.R. § 1.121(c)(1)(ii)

1. (Amended) [Process] A process for the preparation of poly(hydroxy fatty acids) comprising incubating a recombinant organism in a mineral medium under aerobic conditions, expressing [with at least one subunit by means of recombinant bacteria which contain and express] at least one fragment of the gene [of] encoding the poly(hydroxy fatty acid) synthase from *Thiocapsa pfennigii* with a substrate carbon source, wherein the recombinant organism produces a poly(hydroxy fatty acid) and,

the poly (hydroxy fatty acid) is recovered. [and which are selected from the group comprising:

Pseudomonas putida GPp104 (pHP1014::E156), Alcaligenes eutrophus PHB 4 (pHP1014::EIS6), Pseudomonas putida GPp104 (pHP1014::B28+) [DSM #9417] and Alcaligenes eutrophus PHB 4 (pHP1014:B28+) [DSM # 9418], whereby the bacteria are cultivated in a mineral medium under aerobic conditions, whereby

•one offers the bacteria at least one substrate carbon source which is selected from the group consisting of:

levulinic acid, salts of levulinic acid, esters of levulinic acid, lactones of levulinic acid, substituted levulinic acid or, as the case may be, its derivatives; 5-hydroxyhexanoic acid, its

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salts, esters and lactones; 4-hydroxyoctanoic acid, its salts, esters and lactones; their halogenated derivatives as well as their mixtures;

- •one incubates the bacteria for a certain time with the carbon source; and
- •one isolates the poly(hydroxyl fatty acid) polymers that have been synthesized by the bacteria.]
- 2. (Amended) [Process in accordance with] The process of claim 1, [characterized by the feature that] wherein the bacteria are pre-cultivated in a complex medium.
- [4] <u>3</u>. (Amended) [Process in accordance with] <u>The process of claim 1</u>, [characterized by the feature that] <u>wherein</u> one also adds to the bacterial culture at least one additional carbon source which promotes growth, whereby the carbon source is selected from the group [comprising:] <u>consisting of</u>

citric acid, citric acid salts, citric acid esters and citric acid lactones, octanoic acid, octanoic acid salts, octanoic acid esters, octanoic acid lactones, [and] gluconic acid, gluconic acid salts, gluconic acid esters, gluconic acid lactones, [; their salts, esters and lactones;] hexoses, [especially glucose and fructose; as well as their mixtures] and combinations thereof.

- 4. (Amended) [Process in accordance with] <u>The process of claim 1.</u> [characterized by the feature that] <u>wherein</u> the process is carried out in the form of a batch process, a fed-batch process, a two-step process or a continuous flow process.
 - 5. (Amended) [Process in accordance with] The process of claim 1, [characterized

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approximately 15 to 70% by weight [or, especially approximately 15 to 50% by weight or, preferably, approximately 40% by weight] based on the dry mass of the bacterial cells.

- 6. (Amended) [Process in accordance with] The process of claim 1, [characterized by the feature that] wherein the poly(hydroxy fatty acids) are obtained in the form of copolyesters with at least two [or preferably, three] subunits.
- 7. (Amended) [Process in accordance with] The process of claim 1, [characterized by the feature that] wherein the recombinant bacteria are cultivated at cell densities of up to 100 g of dry cellular mass per liter of bacterial nutrient medium.
- 8. (Amended) [Process in accordance with] <u>The process of [claims] claim 1</u>, [characterized by the feature that] <u>wherein</u> one offers the substrate carbon source in excess.
- 9. (Amended) [Process in accordance with] The process of claim 8, [characterized by the feature that] wherein one [uses] offers the substrate carbon source at a concentration of approximately 0.1 to 5% by weight.
- 10. (Amended) [Process in accordance with] The process of claim 9, [characterized by the feature that] wherein one increases the concentration of the substrate carbon source in the culture medium in steps, optionally with pre-cultivation in the presence of an additional carbon source which does not serve as a substrate.
- 11. (Amended) [Process in accordance with] The process of claim 10, [characterized by the feature that] wherein, [in each case], one adds approximately 0.5% (weight/volume) of

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neutralized substrate carbon source after approximately 12 h and 24 h at approximately 27°C to 35°C [or, preferably, at approximately 30°C].

- 12. (Amended) [Process in accordance with] The process of claim 1, [characterized by the feature that] wherein cultivation takes place for approximately 24 h to 96 h [or, especially, for approximately 36 h to 72 h or, preferably, for approximately 48 h to 72 h].
- by the feature that] wherein the recombinant bacteria are cultivated under conditions deficient in an element [of deficiency, preferably under] wherein the element is selected from the group consisting of [conditions of a deficiency of] nitrogen, magnesium or phosphate.
- by the feature that] wherein the harvested recombinant bacteria are broken open [by means of physical and/or chemical and/or biochemical processes] in order to obtain the poly(hydroxy fatty acids) that have been produced [bio-technically].
- 15. (Amended) [Process in accordance with] The process of Claim 14, [characterized by the feature that] wherein the harvested recombinant bacteria are lyophilized and then extracted with an organic solvent. [preferably] selected from the group consisting of chloroform or methylene chloride, in order to break open the recombinant bacteria and to obtain the poly(hydroxy fatty acids).
 - 16. (Amended) [Process in accordance with] The process of claim 15, [characterized

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introducing a hydrophilic solvent, [especially] <u>selected from the group consisting of water [or] and a lower alcohol, [preferably ethanol,] [and] wherein the product is obtained in essentially pure form by removing the hydrophilic solvent.</u>

- by the feature that] wherein the harvested recombinant bacteria are broken open by means [of] selected from the group consisting of detergents, [and/or] a lytic enzyme cocktail, and a combination thereof [as a result of which] wherein the bacterial cell grana, which contain the poly(hydroxy fatty acid), sediment to the bottom of the bio-reactor and are collected from there in order to be processed further.
- 18. (Amended) [Process in accordance with] <u>The process of claim 17</u>, [characterized by the feature that] <u>wherein</u> the lytic enzyme cocktail contains enzymes which are selected from the group [which comprises:] <u>consisting of</u>

lysozyme; proteases; other hydrolytic enzymes; [as well as their mixtures] and combinations thereof.

Please add the following new claims.

- 37. The process of claim 1, wherein the poly(hydroxy fatty acid) is obtained in a concentration of approximately 15 to 50% by weight based on the dry mass of the bacterial cells.
- 38. The process of claim 1, wherein the poly(hydroxy fatty acid) is obtained in a concentration of approximately 40% by weight based on the dry mass of the bacterial cells.

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39. The process of claim 10, wherein, in each case, one adds approximately 0.5% (weight/volume) of neutralized substrate carbon source after approximately 12 h and 24 h at approximately 30°C. 40. The process of claim 1, wherein cultivation takes place for approximately 36 h to 72 h. The process of claim 1, wherein cultivation takes place for approximately 48 h to 72 h. The process of claim 1 wherein the poly(hydroxy fatty acid) polymer is comprised 42. of one or more monomers selected from the group consisting of: 3-hydroxybutyric acid, 3 hydroxyvaleric acid and 4-hydroxy-valeric acid; 3-hydroxybutyric acid, 3 hydroxyvaleric acid, 4-hydroxy-valeric acid, 3hydroxyhexanoic acid and 3-hydroxyoctanoic acid; 3-hydroxybutyric acid, 3-hydroxyhexanoic acid, 5-hydroxyhexanoic acid, and 3hydroxyoctanoic acid; 3-hydroxybutyric acid, 3 hydroxyvaleric acid, 3-hydroxyhexanoic acid, 3hydroxyheptanoic acid, 4-hydroxyheptanoic acid and 3-hydroxyoctanoic acid; 3-hydroxybutyric acid, 3 hydroxyhexanoic acid, 3-hydroxy-octanoic acid and 4hydroxyoctanoic acid; 3-hydroxybutvric acid. 3-hydroxybexanoic acid and 5-hydroxybexanoic acid;



CLEAN VERSION OF AMENDED SPECIFICATION PARAGRAPHS PURSUANT TO 37 C.F.R. § 1.121

Clean Version of Amended Specification Paragraphs Pursuant to 37 C.F.R. § 1.121(b)(1)(ii)

Please replace the paragraph bridging pages 1 and 2 with the following paragraph.

-- The present invention relates to a process for the production of poly(hydroxyl acids) by means of recombinant bacteria which contain and express at least one fragment of the gene of poly(hydroxy fatty acid) synthase from *Thiocapsa pfennigii* and which are selected from the group comprising: *Pseudomonas putida* GPp104 (pHP1014::E156), *Alcaligenes eutrophus* PHB 4 (pHP1014::E156), *Pseudomonas putida* GPp104 (pHP1014::B28+) [DSM # 9417] and *Alcaligenes eutrophus* PHB 4 (pHP1014:B28+) [DSM # 9418], whereby the bacteria are cultivated in a mineral medium under aerobic conditions, whereby one offers the bacteria at least one substrate carbon source which is selected from the group consisting of: levulinic acid, salts of levulinic acid, esters of levulinic acid, lactones of levulinic acid, substituted levulinic acid or, as the case may be, its derivatives: 5-hydroxyhexanoic acid, its salts, esters and lactones; 4-hydroxyheptanoic acid, its salts, esters and lactones; their halogenated derivatives as well as their mixtures; one incubates the bacteria for a certain time with the carbon source; and one isolates the poly(hydroxyl fatty acid) polymers that have been synthesized by the bacteria:

a recombinant bacterial strain characterized by the feature that the bacterial strain is selected from the group which comprises *Pseudomonas putida* GPp104 (pHP1014::B28+) [DSM # 9417] and *Alcaligenes eutrophus* PHB 4 (pHP1014::B28+) [DSM # 9418];

a poly(hydroxyl fatty acid) produced by any one of the previously described processes:

and a DNA fragment which codes for a pha E component and a pha C component of the poly(hydroxyl fatty acid) synthase from *Thiocapsa pfennigii* characterized by the feature that it has at least the nucleotide sequence of

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CLEAN VERSION OF AMENDED SPECIFICATION PARAGRAPHS

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sequence sections 180 through 1280 (phaE) and 1322 through 2392 (phaC) of the DNA sequence SEQ ID NO:1.--

Please replace the paragraph bridging pages 10 and 11 with the following paragraph.

-- From a process technical standpoint, the above problem is solved by a process for the preparation of poly(hydroxy fatty acids) with at least one subunit by means of recombinant bacteria which contain and express at least one fragment of the gene of poly(hydroxy fatty acid) synthase from Thiocapsa pfennigii and which are selected from the group comprising: Pseudomonas putida GPp104 (pHP1014::E156), Alcaligenes eutrophus Pseudomonas putida GPp104 (pHP1014::B28+) [DSM #9417] and (pHP1014::E156), Alcaligenes eutrophus PHB 4 (pHP1014:B28+) [DSM # 9418], whereby the bacteria are cultivated in a mineral medium under aerobic conditions, whereby one offers the bacteria at least one substrate carbon source which is selected from the group consisting of: levulinic acid, salts of levulinic acid, esters of levulinic acid, lactones of levulinic acid, substituted levulinic acid or, as the case may be, its derivatives; 5-hydroxyhexanoic acid, its salts, esters and lactones; 4hydroxyheptanoic acid, its salts, esters and lactones; 4-hydroxyoctanoic acid, its salts, esters and lactones; their halogenated derivatives as well as their mixtures; one incubates the bacteria for a certain time with the carbon; and one isolates the poly(hydroxy fatty acid) polymers that have been synthesized by the bacteria.--

Please replace the paragraph bridging pages 11 and 12 with the following paragraph.

-- In accordance with the process for the preparation of poly(hydroxy fatty acids) with at least one subunit by means of recombinant bacteria which contain and express at least one fragment of the gene of poly(hydroxy fatty acid) synthase from *Thiocapsa pfennigii* and which are selected from the group comprising: *Pseudomonas putida* GPp104 (pHP1014::E156).

 $I = CD_{22}IOA$

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9418], whereby the bacteria are cultivated in a mineral medium under aerobic conditions, whereby one offers the bacteria at least one substrate carbon source which is selected from the group consisting of: levulinic acid, salts of levulinic acid, esters of levulinic acid, lactones of levulinic acid, substituted levulinic acid or, as the case may be, its derivatives; 5-hydroxyhexanoic acid, its salts, esters and lactones; 4-hydroxyheptanoic acid, its salts, esters and lactones; 4-hydroxyoctanoic acid, its salts, esters and lactones; their halogenated derivatives as well as their mixtures; one incubates the bacteria for a certain time with the carbon source; and one isolates the poly(hydroxy fatty acid) polymers that have been synthesized by the bacteria, it has been possible for the first time to produce 4HV-containing polyesters starting out from levulinic acid. The chemical structure of levulinic acid is reproduced in the following formula:--

ATL1 #553905 v1